1652
#13
4/25/03

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Wezel et al.

Serial No.: 09/749,185

Filed: December 26, 2000

For: REDUCING BRANCHING AND ENHANCING FRAGMENTATION IN CULTURING FILAMENTOUS MICROORGANISMS

Confirmation No.: 6157

Examiner: M. Rao

Group Art Unit: 1652

Attorney Docket No.: 2183-4666US

CERTIFICATE OF MAILING

I hereby certify that this correspondence along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service as First Class Mail on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231.

April 16, 2003
Date

Betty Vowles
Signature

Betty Vowles
Name (Type/Print)

COMMUNICATION

Commissioner for Patents
Washington, D.C. 20231

Sir:

Enclosed is a Priority Document 98202148.7 filed 26 June 1998 for the above-referenced application.

Respectfully submitted,

Allen C. Turner
Registration No. 33,041
Attorney for Applicant(s)
TRASKBRITT, P.C.
P.O. Box 2550
Salt Lake City, Utah 84110-2550
Telephone: 801-532-1922

Date: April 15, 2003
ACT/bv
Document in ProLaw

Eur päisches Patentamt
Eur pean Patent Office
Office eur péen des brevets

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

98202148.7

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

DEN HAAG, DEN
THE HAGUE, 24/03/03
LA HAYE, LE

Europäisches Patentamt | European Patent Office | Office européen des brevets

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.: Application no.: Demande n°: 98202148.7

Anmeldetag: Date of filing: Date de dépôt: 26/06/98

Anmelder: Applicant(s): Demandeur(s):
Rijksuniversiteit te Leiden
2312 AV Leiden
NETHERLANDS

Bezeichnung der Erfindung: Title of the invention: Titre de l'invention:
Reducing branching and enhancing fragmentation in culturing filamentous microorganisms

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Internationale Patentklassifikation: International Patent classification: Classification internationale des brevets:
C12N15/31, C12N5/10, C07K14/36

Am Anmeldetag benannte Vertragstaaten: Contracting states designated at date of filing: Etats contractants désignés lors du depôt: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE

Bemerkungen: Remarks: Remarques:

Title: Reducing branching and enhancing fragmentation in culturing filamentous microorganisms

The invention relates to industrial microbiology, in particular to fermentation technology and especially to fermentation methods for filamentous microorganisms, in particular filamentous bacteria such as actinomycetes. The invention was made in a research program into mechanisms of growth of streptomycetes.

Streptomycetes are Gram-positive, aerobic, filamentous soil bacteria, which belong to the order of actinomycetales. In an early stage of *Streptomyces* growth on a solid medium, spores germinate, and subsequently develop into a vegetative mycelium of branching hyphae (Chater and Losick, 1996). After environmental signals such as nutrient depletion, aseptate aerial hyphae are formed, growing on the vegetative hyphae, the latter being used as a substrate. Eventually, the aerial hyphae form uninucleoid cells that develop into hydrophobic spores, which are budded off from the tips of the hyphae. One of the striking features of streptomycetes and other members of the order of actinomycetales is their ability to produce a wide variety of secondary metabolites, including many antibiotics, which are produced in temporal relation to the onset of morphological differentiation in surface-grown cultures (Chater, 1989; Miyadoh, 1993). The molecular processes regulating the events that lead to differentiation of *Streptomyces* are presently only superficially understood, although new and interesting insights into the genetics of streptomycetes have come to light (reviewed in Champness and Chater, 1993; Chater, 1993).

Most streptomycetes only sporulate on solid media, while growth in liquid cultures is restricted to the formation of vegetative mycelium. This typically develops into intricate networks of hyphae, among others resulting in pellet formation, with only the most outwardly oriented

sections showing high physiological activity, resulting in low yield of the desired product per unit of biomass. Furthermore, because of their filamentous morphology, high density fermentations of biotechnologically interesting streptomycetes often are highly viscous, resulting in a low biomass accumulation due to for instance aeration and mixing problems. From this perspective it is desirable that fragmentation of the mycelium in submerged cultures is stimulated, that branching of the mycelium is reduced and that in general the viscosity of the culture is reduced.

Cell division in all bacteria analysed so far involves the tubulin-like GTP-binding protein FtsZ, which polymerises into a ring at the prospected site of the septum, presumably forming the physical scaffold for the assembly of the cell division apparatus (reviewed in Lutkenhaus and Addinall, 1997). In *Escherichia coli* and *Bacillus* species many factors have been identified that are involved in cell division, but little is known about this process in actinomycetes. Here septum formation does not lead to actual cell division, and while in most bacteria ftsZ is essential, the gene has been shown to be dispensable for mycelial growth in *Streptomyces coelicolor* (McCormick et al., 1994).

In contrast to most actinomycetes, *Streptomyces griseus* shows the ability to sporulate in submerged cultures over a short time period, when grown in defined minimal media (Kendrick and Ensign, 1983; Ensign, 1988). Kawamoto and Ensign (1995a,b) identified a mutation in the gene *ssgA* that relieved repression of sporulation in rich media. *SsgA* encodes an acidic protein with a molecular mass of approximately 16 kDa that displays no significant homology to any known protein in the database. Furthermore, overexpression of *ssgA* resulted in fragmented growth and suppression of sporulation in submerged cultures of *S. griseus*. Western blot analysis with polyclonal antibodies

raised against SsgA revealed that expression of SsgA directly correlates to the onset of submerged sporulation, with the protein appearing shortly before spore formation (Kawamoto et al., 1997). Importantly, although sporulation and production of the antibiotic streptomycin are apparently linked in *S. griseus*, no suppression of streptomycin production was observed. Apparently, regulation of sporulation and antibiotic biosynthesis occur via separate pathways.

The present inventors have shown that the activity of SsgA is not limited to the organism in which it is found. The activity can advantageously be transferred to other organisms, thereby allowing more fragmented growth and/or reduced branching and/or reduced viscosity of the culture of many filamentous microorganisms, in particular actinomycetes and Steptomycetes. It is particularly surprising that organisms that do not even harbour an *ssgA*-like gene themselves still respond to the presence of the product of such a gene. Thus we demonstrate that introduction of *ssgA* into various bacteria, in particular actinomycetes that do not harbour an endogenous *ssgA*, results in suppressed branching and enhanced fragmentation of the mycelium in liquid culture, resulting in significantly lower viscosity of culture broths. In addition to autonomously replicating plasmids containing constitutively expressed *ssgA*, we devised a system that allows easy integration of the gene in the chromosome, with the advantage of high stability.

Thus the invention now provides a method for producing a filamentous bacterium showing reduced branching during growth, particularly growth in a liquid medium, comprising providing such a bacterium with the capability of having or expressing heterologous SsgA-activity, which activity in *Streptomyces Griseus* is encoded by an *ssgA* gene having the sequence.

1 ATGCGCGAGTCGGTTCAAGCAGAGGTCATGATGAGCTTCCTCGTCTCCGA

51 GGAGCTCTCGTTCCGTATTCCGGTGGAGCTCCGATACGAGGTCGGCGATC

101 CGTATGCCATCCGGATGACGTTCCACCTTCCCGGCGATGCCCCTGTGACC

151 TGGGCGTTCGGCCGCGAGCTGCTGCTGGACGGGCTCAACAGCCCGAGCGG

201 CGACGGCGATGTGCACATCGGCCCGACCGAGCCCGAGGGCCTCGGAGATG

251 TCCACATCCGGCTCCAGGTCGGCGCGGACCGTGCGCTGTTCCGGGCGGGG

301 ACGGCACCGCTGGTGGCGTTCCTCGACCGGACGGACAAGCTCGTGCCGCT

351 CGGCCAGGAGCACACGCTGGGTGACTTCGACGGCAACCTGGAGGACGCAC

401 TGGGCCGCATCCTCGCCGAGGAGCAGAACGCCGGCTGA

As explained above the presence of additional SsgA-activity, in particular heterologous SsgA-activity (meaning activity not in a form as present in the microorganism in nature) leads to enhanced fragmentation, reduced branching and thus reduced viscosity. The activity may be provided in any suitable manner, but it is preferred that the activity is provided by transfecting or transforming said filamentous bactrium with additional genetic information encoding said activity. Examples of such methods are presented hereinbelow, but the art of genetic engineering of bacteria is so well advanced that persons skilled in the art will be able to come up with numerous methods and variations thereof to provide a intended filamentous bacterium with a gene encoding SsgA-like activity. SsgA-like activity is functionally defined as the ability to enhance fragmentation and/or reduce branching in (typically) submerged cultures of filamentous microorganisms,

in particular bacteria, more specifically actinomycetes. The activity of other *ssgA*-like genes or fragments of *ssgA* genes or derivatives of *ssgA* genes which are within the invention must be functionally the same, but that does not mean that the amount of activity per molecule needs to be the same. SsgA-like activity is thus defined as similar in kind, though not necessarily in amount. Other genes encoding such SsgA activity than the genes disclosed herein can be obtained without departing from the invention by applying routine hybridization and/or amplification techniques. Means and methods for expressing such genes are well known in the art so that there is no need to go into detail here regarding cloning vectors, expression vectors, (inducible) promoters, enhancers, repressors, restriction enzymes, etc. etc. For stability of the presence of the added SsgA-activity to the bacterium, in particular for application in large scale fementations, it is however preferred that the genetic information encoding the additional SsgA activity is integrated into the host cell genome. In this case typically the genetic information will be in the form of DNA. However, neither RNA, heteroduplexes or even PNAs are excluded from the present invention as means to provide the additional genetic information to a microorganism. The invention is preferably applied in the field of filamentous bacteria, in particular actinomycetes and most specifically to streptomycetes. In these embodiments in particular it is preferred to apply *ssgA* genes derived from actinomycetes, especially from other actinomycetes than the one to be altered in growth characteristics. This of course is automatically the case in a bacterium that does not have SsgA activity to any significant amount itself. Using a gene from a related organism enhances the compatibility of the expression machinery of the host with the gene. Thus it is particularly preferred to provide a *Streptomyces* with an *ssgA*

(-like) gene from a different *Streptomyces*. *SsgA* genes are found in *Streptomyces griseus*, *Streptomyces collinus*, *Streptomyces albus*, *Streptomyces goldeniensis* and *Streptomyces netropsis*. It is preferred to provide *Streptomyces* strains not having such a gene with a gene from the earlier mentioned strains.

Typically said filamentous bacterium not having such a gene does not have significant endogenous SsgA-activity.

It is useful to ensure that said additional SsgA-activity is inducible or repressible with a signal. In this way the growth characteristics of the bacteria can be modified at will. Of course the final goal of the present invention is to enhance the production of useful products by the microorganisms by modifying the microorganisms according to the invention. Useful products produced by or through microorganisms according to the invention include so called secondary metabolites, typically antibiotics or antitumour agents, but also immunosuppressive agents, hypocholesterolemic agents, enzyme inhibitors, antimigraine agents, herbicides, antiparasitic agents, ruminant growth promoters, bioinsecticides, receptor (ant)agonists, hetreolgous proteins or even simple biomass. In the case of Streptomycetes such a useful product is typically an antibiotic. It is thus therefore preferred according to the invention to modify antibiotic producing strains of *Streptomyces*, particularly those not harbouring an *ssgA*-like gene, with genetic information encoding ssgA activity. On the other hand the invention can also be very suitably applied to Streptomycetes or other microorganisms expressing heterologous proteins (or overexpressing homologous/endogenous proteins).

For ease of production it is preferred that the useful product, said antibiotic or said protein, is secreted by said bacterium. The protein to be expressed may very well

be a protein involved in the pathway of making a useful product such as an antibiotic, so that this production can be further enhanced on top of the improvement by the reduced fragmentation, etc. In that case it would be very suitable to combine the two genes on one vehicle for introduction into the bacterium. The bacteria resulting from the methods according to the invention are of course also part of the invention. They have additional SsgA-activity (or are capable of expressing such activity) and they thereby will typically have different growth characteristics than the unmodified microorganisms when said SsgA activity is present. Thus the invention also provides a filamentous bacterium obtainable by a method according to invention. Preferred microorganisms according to the invention are actinomycetes and typically streptomycetes. As stated above it is an important goal of the present invention to improve fermentative production of useful products such as antibiotics. Thus the invention also provides a method for producing an antibiotic or a useful protein comprising culturing a filamentous bacterium according to the invention and harvesting said antibiotic or protein from said culture. The advantages of the invention are most clear when the method of culturing is submerged culture. The invention will be explained in more detail in the following experimental part.

Experimental procedures

Bacterial strains, culture conditions and plasmids

E. coli K-12 strains JM109 (Messing *et al.*, 1981), and ET12567 (MacNeil, *et al.*, 1992) were used for routine sub-cloning. The strains were grown and transformed by standard procedures (Sambrook *et al.*, 1989); transformants were selected in L broth containing 1% (w/v) glucose, and ampicillin at a final concentration of 200 µg ml^{-1}. L broth

with 1% glucose and 30 μg ml^{-1} chloramphenicol was used to grow ET12567.

Streptomyces coelicolor A3(2) M145 and *Streptomyces lividans* 1326 (Hopwood *et al.*, 1985) were used for transformation and propagation of *Streptomyces* plasmids. Protoplast preparation and transformation were performed as described by Hopwood *et al.* (1985). SFM medium (mannitol, 20 g l^{-1}; soya flour, 20 g l^{-1}; agar, 20 g l^{-1}, dissolved in tap water) is a modified version of that reported by Hobbs *et al.* (1989) and was used to make spore suspensions. R2YE (Hopwood *et al.*, 1985) was used for regenerating protoplasts and, after addition of the appropriate antibiotic, for selecting recombinants.

For liquid culturing of *Streptomyces* we used YEME medium (Hopwood *et al.*, 1985), Tryptone soy broth (Difco) containing 10% sucrose (designated TSBS), or standard minimal medium (MM; Hopwood *et al.*) with 1% mannitol as carbon source.

Strains used for screening of *ssgA* were *Streptomyces albus* G (ATCC 3004), *Streptomyces ambofaciens* (ATCC 23877), *Streptomyces antibioticus* (ATCC8663), *Streptomyces clavuligerus* (ATCC 27064), *Streptomyces coelicolor* M145, *Streptomyces collinus* (DSM 40733), *Streptomyces fradiae* (CBS 498.68), *Streptomyces goldeniensis* (ATCC 21386), *Streptomyces griseus* (ATCC 23345), *Streptomyces kasugaensis* (DSM 40819), *Streptomyces lividans*, *Streptomyces mobaraensis* (ATCC 25365), *Streptomyces netropsis* (formerly *Streptoverticilium netropsis*; ATCC 23940), *Streptomyces ramocissimus* (ATCC 27529), and the actinomycetes *Nocardia lactamdurans* (ATCC 27382), *Planobispora rosea* (ATCC 53773), *Saccharopolyspora erythraea* (NRRL 2338).

Plasmids pUC18 (Yanisch-Perron *et al.*, 1985), pIJ2925 (Janssen and Bibb, 1993), and pSET152 (Bierman *et al.*, 1992)

were used for cloning experiments. While pSET152 is a conjugative shuttle plasmid, in the experiments described in this study the plasmid and its derivatives were introduced by standard protoplast transformation.

pIJ486 (Ward *et al.*, 1986) and the *E. coli/Streptomyces* shuttle vector pWHM3 (Vara *et al.*) as high copy-number vectors (approximately 50-100 copies per chromosome) in *S. coelicolor*. An expression vector, designated pWHM3-E, was constructed by cloning the 300 bp EcoRI/BamHI fragment containing the *ermE* promoter (Bibb *et al.*, 1994) into pWHM3. Standard procedures were used to isolate plasmid DNA from *E. coli* (Sambrook *et al.*, 1989), and to isolate plasmid and total DNA from *Streptomyces* (Hopwood *et al.*, 1985).

PCR conditions

Polymerase chain reactions (PCRs) were performed in a minicycler (MJ Research, Watertown, MA), using Pfu polymerase (Stratagene, La Jolla, LA), and the buffer provided by the supplier, in the presence of 5% (v/v) DMSO and 200 mM dNTP. No additional Mg^{++} was added to the reaction mixture. The following PCR program was used: 30 cycles of 45 seconds melting at 94°C, 1 minute annealing at 54°C, and 90 seconds extension at 72°C, followed by an additional 10 minutes at 72°C.

Constructs for expression of *ssgA*

A 750 bp DNA fragment containing the *ssgA* gene (Accession D50051) was amplified from the *Streptomyces griseus* chromosome by PCR, using primers ssg1 and ssg2 (Table 1). The PCR fragment was cloned as an *Eco*RI-*Bam*HI fragment in pIJ2925, and further into pWHM3, pWHM3-E, and pSET152, resulting in pGWS1, pGWS2, pGWS3, and pGWS4, respectively

(Table 1). For pGWS1 and pGWS3, see also Figure 1. The *S. coelicolor* strain with pGWS4 integrated in the *attP* site on the chromosome was designated *S. coelicolor* GSA1. For pGWS1, pGWS3, and pGWS4 we also made derivatives in which the upstream region of *S. griseus ssgA* was replaced by that of *S. ramocissmus tuf1* (Vijgenboom *et al.*, 1994), which is known to be very efficiently recognized by ribosomes and hence typically results in higher expression; these were designated pGWS1-SD, pGWS3-SD, and pGWS4-SD, respectively.

Southern hybridization and probes

Genomic DNAs used for Southern analysis were isolated according to the method described by Hopwood *et al.* (1985). For high-resolution hybridization experiments, to investigate the presence of *ssgA* in various actinomycetes, genomic DNA was digested with the appropriate enzymes and separated electrophoretically on a 0.7% agarose gel in TAE buffer, using the Gibco BRL 1 kb ladder as DNA size markers. Agarose gels were pretreated and subsequently blotted on Hybond-N^+ nylon membranes (Amersham) using 20x SSC buffer as the transfer buffer, basically according to Sambrook *et al.* (1989). Hybridization and washing conditions were described previously (van Wezel *et al.*, 1991). Stripping of blots was done by 30 minutes incubation in 0.4 N NaOH at 65°C and subsequent incubation in 0.1x SSC/0.25 M Tris (pH 6.5). The total removal of the probe was checked by overnight exposure of an X-ray film.

For recognition of *ssgA* in Southern hybridization experiments the 580 bp insert from pGWS5 was [^{32}P]-labelled by the random-prime method (Feinberg and Vogelstein, 1983).

Computer analysis

The BLAST search engines BlastN, BlastP, and BlastX (Altschul *et al.*, 1990) were used to perform database searches, and the Wisconsin GCG Package (Devereux *et al.*, 1984) for sequence alignments and protein analysis.

Results

SsgA is a unique protein that does not belong to any known protein family

Extensive searches with *S. griseus* SsgA of both the translated nucleotide database and the protein database using the BLAST search engines BLASTX and BLASTP resulted in one relevant hit, namely a partial sequence of *Streptomyces albus* G DNA (Accession M28303) that apparently encodes part of SsgA. This DNA was identified upstream of a ß-lactamase gene (Dehottay *et al.*, 1987), and apparently encodes 67 residues of a putative protein with 86% aa identity to aa 18-84 of *S. griseus* SsgA. The lack of the C-terminal half of the gene suggests that the cloning of this *ssgA* homologue was probably coincidental and the result of a cloning artifact. The cloning and sequencing of the complete gene is described below.

Cloning of *S. griseus ssgA* by PCR

The sequence of *S. griseus ssgA* was published by Kawamoto and Ensign (1995b), and deposited in the EMBL/GENBANK database (D50051). In a recent update the translational start codon was proposed 30 nt downstream of the originally indicated start codon. This ambiguity does not influence the outcome of our experiments. Based on protein electrophoresis (SDS PAGE) experiments using over-expressed SsgA, we believe that the second (further downstream located)

ATG triplet represents the correct translational start codon (data not shown).

The 750 bp DNA fragment generated by PCR amplification of *S. griseus* chromosomal DNA using oligonucleotides ssg1 and ssg2 was cloned into pIJ2925, resulting in pGWS1 (Table 1). Restriction site and sequence analysis confirmed that the fragment indeed contained *ssgA*.

Southern hybridization reveals *ssgA* in a limited number of streptomycetes

Genomic DNAs isolated from several actinomycetes (see legend to Fig. 2) was digested with *Bam*HI and *Pst*I, submitted to agarose gel electrophoresis and hybridised with the 580 bp insert from pGWS5 harbouring *S. griseus ssgA*, under conditions of low stringency to identify all genes with at least remote similarity to *ssgA*. One hybridising band was observed in the lanes containing *S. collinus*, *S. albus*, *S. goldeniensis*, and *S. griseus* genomic DNAs, and two bands of equal intensity in the lane containing *S. netropsis* DNA (Fig. 2). We failed to detect a band corresponding to *ssgA* in all other *Streptomyces* species, including *S. coelicolor* and *S. lividans*, in contrast to a previous Southern analysis by Kawamoto and Ensign (1995b), who used a probe that included *ssgA* flanking sequences. The duplicity of the signal corresponding to *ssgA* in *S. netropsis* was due to a *Bam*HI restiction site in the gene, as can be deduced from the DNA sequence. We also could not detect an *ssgA* homologue in any of the other actinomycetes checked, namely *Nocardia lactamdurans*, *Planobispora rosea*, and *Saccharopolyspora erythraea*.

Cloning and sequencing of *ssgA* homologues from other streptomycetes

Genomic DNA fragments harbouring *ssgA* homologues from three streptomycetes, namely *S. albus*, *S. goldeniensis*, and *S. netropsis*, were amplified by PCR, using oligonucleotides ssg3 and ssg4. These fragments were cloned as *Eco*RI/*Bam*HI fragments into pIJ2925, and the DNA sequence was determined. Table 2 shows the similarities of the *ssgA* genes and the deduced amino acid sequences. Interestingly, the *S. netropsis* and *S. griseus ssgA* gene products share more than 86% identical amino acids (90% similar), which is high in comparison to 79% (85%) for *S. goldeniensis* SsgA and, strikingly, a poor 63% (71%) for *S. albus* SsgA.

***S. griseus* and *S. netropsis* sporulate in liquid cultures**

The morphology of the streptomycetes and actinomycetes discussed in this paper was checked by light microscopy. To this purpose, the strains were grown in complex (TSBS) or minimal (MM) liquid medium for three days, and growth characteristics monitored. From these experiments it appeared that only S. *griseus* and *S. netropsis* produced abundant spores in liquid cultures, while *S. goldeniensis* and *S. collinus* showed unusual thickening of the tips of the hyphae, but failed to sporulate under the chosen conditions. Interestingly, while *S. griseus* sporulated only in MM, as was already reported by Kendrick and Ensign (1983), *S. netropsis* sporulated abundantly in TSBS as well as in MM. We believe that the relation between sporulation and (the amino acid sequence of) SsgA is of particular interest.

Expression of *ssgA* in *S. coelicolor* M145 results in reduced branching of the hyphae and fragmented growth

The insert of pGWS1 was cloned into pWHM3 and pWHM3-E, multicopy shuttle vectors that replicate in *E. coli* and *Streptomyces*. The resulting plasmids pGWS2 and pGWS3 (Table 1) were introduced into *S. coelicolor* M145 and correct recombinants were selected by checking the insert lengths of the plasmids. In a control experiment we used pWHM3-E transformants.

Transformants containing pWHM3-E (without *ssgA*) or pGWS2 showed little or no altered morphology in the complex liquid media TSBS, YEME, nor in minimal medium (MM), as judged by phase-contrast microscopy (Fig. 3A). However, hyphae of transformants containing pGWS3 showed strongly reduced branching in complex and minimal medium cultures, resulting in clearly less dense mycelial lumps (Fig. 3B). The vegetative hyphae not only show limited branching, but many of the branches are less than a micron in length. When pGWS3-SD was used instead of pGWS3, the effect was even stronger, with small fragments appearing after approximately 30 hrs, which increased over time (Fig. 4). While MM cultures of *S. coelicolor* typically result in very large mycelial lumps that sediment rapidly (virtually all mycelium precipitates within one minute when shaking was stopped), MM cultures containing pGWS3-SD transformants showed significantly reduced sedimentation rates, with the majority of the mycelium failing to sediment within five minutes after shaking of the cultures was stopped.

Expression of chromosomally-integrated *ssgA* also results in fragmented growth

The insert of pGWS3 and pGWS3-SD was cloned in pSET152, a conjugative *E. coli/Streptomyces* shuttle vector,

resulting in pGWS4 and pGWS4-SD, respectively. These plasmids were introduced into *S. coelicolor* M145 by standard protoplast transformation, and transformants selected by overlay of the transformation plates with apramycin. Chromosomal integration was checked by Southern analysis, and presence of the complete gene confirmed by PCR using oligonucleotides ssg1 and ssg2. The pGWS4 and pGWS4-SD integrants were designated GSA1 and GSA2. *S. coelicolor* M145 harbouring pSET152 without *ssgA* was used as control strain.

While recombinants containing pSET152 displayed wild-type phenotype, with large mycelial lumps and very few smaller fragments, GSA1 showed limited branching, while the phenotype of GSA2 is much similar to that of *S. coelicolor* harbouring pGWS3-SD, with strongly limited branching and fragmented growth (Fig. 4). This shows that *S. griseus ssgA* integrated in the *S. coelicolor* chromosome can be expressed at a level high enough to allow fragmentation of *S. coelicolor* mycelium in complex and minimal liquid cultures.

High level expression of *ssgA* in other actinomycetes

The *ssgA* expression vectors pGWS3-SD and pGWS4 were introduced in *S. lividans*, *S. clavuligerus*, and *Sacch. erythraea*, to test the effect of SsgA on the morphology of strains other than *S. coelicolor*. Expression in *S. lividans* using pGWS3-SD or pGWS4 led to a phenotype much similar to that of *S. coelicolor* harbouring the same plasmids, as was expected since *S. lividans* and *S. coelicolor* are strongly related streptomycetes. Interestingly, expression of SsgA in both *S. clavuligerus* and *Sacch. erythraea* also resulted in reduced branching and increased fragmentation in liquid cultures, even though morphology of these strains is different from that of *S. coelicolor*.

Thus, it appears that overproduction of SsgA has a strong effect on mycelium morphology in submerged cultures of not only *S. coelicolor*, but also of *S. lividans*, *S. clavuligerus*, and *Sacch. erythraea*, with the vegetative hyphae showing restricted branching. Furthermore, the ageing cultures showed an increasing degree of fragmentation, resulting in higher culture densities and lower viscosity of recombinant streptomycetes expressing SsgA. Comparison of the phenotypes of the two categories of *Streptomyces* strains, namely those possessing *ssgA* and those lacking *ssgA*, is currently in progress, and could give us more insight into the role of SsgA in *Streptomyces* physiology.

Figure legends

Figure 1. Some of the *ssgA* constructs. Arrows show direction of *ssgA*. P_{ermE}, *ermE* promoter; P_{T7}, T7 promoter. Solid lines represent *ssgA* DNA, broken lines represent plasmid DNA.

Figure 2. Southern hybridization for the detection of *ssgA* in actinomycetes. All numbered lanes contain *Bam*HI/*Pst*I-digested chromomal DNA. Marker lanes (M) contain 1 kb DNA ladder. Blots were hybridized with the 580 bp insert from pGWS5 as probe, and subsequently with a small amount of radioactively labelled 1 kb ladder.

A. Lanes: 1. *S. coelicolor* 2. *S. lividans* 1326 3. *S. lividans* TK24 4. *S. griseofuscus* 5. *S. fradiae* 6. *S. ramocissimus* 7. *S. collinus* 8. *S. kasugaensis* 9. *S. antibioticus* 10. *Sacch. erythraea* 11. *N. lactamdurans* 12. *P. rosea* 13. *S. griseus*

B. Lanes: 1. *S. albus* 2. *S. ambofaciens* 3. *S. coelicolor* 4. *S. clavuligerus* 5. *S. collinus* 6. *Sacch. erythraea* 7. *S. goldeniensis* 8. *S. mobaraensis* 9. *S. netropsis* 10. *P. rosea*

Figure 3. Phase-contrast microscopy of *S. coelicolor* M145 containing pGWS2, pGWS3, and pGWS3-SD.

Figure 4. Phase-contrast microscopy of *S. coelicolor* M145 containing chromosomally-integrated pGWS4.

Figure 5. Sequences of different *ssga* genes and proteins from different strains and oligonucleotides.

Oligonucleotides

primer		Nucl. Pos.
ssg1	5' GGCGAATTCGAACAGCTACGTGGCGAAGTCGCCA 3' (*Eco*RI)	-194/-170
ssg2	5' GTGGGATCCGTGCTCGCGGCGCTGGTCGTCTC 3' (*Bam*HI)	+539/+517
ssg3	5' GGGAATTCCATATGCGCGAGTCGGTTCAAGCA 3' (*Eco*RI *Nde*I)	-30/-10
ssg4	5' CCGGTCAGCCGGCGTTCTGCTCCTC 3'	+412/388

Plasmids

pIJ2925	Derivative of pUC19, with *Bgl*II sites flanking the slightly altered multiple cloning site.	Janssen and Bibb, 1993
pWHM3	Multi-copy *E. coli/Streptomyces* shuttle vector. Carries thiostrepton resistance marker	Vara *et al.*
pWHM3-E	pWHM3 with the 300 bp fragment containing the constitutive *ermE* promoter for gene expression	this study
pSET152	*E. coli/Streptomyces* shuttle vector that allows integration in the _C31 attachment site on the *Streptomyces* chromosome. Carries apramycin resistance marker.	Bierman *et al.*, 1992
pGWS1	pIJ2925 containing the 750 bp *ssgA* PCR (ssg1/ssg2) product	this study
pGWS1-SD	pGWS1 with the upstream region of *ssgA* replaced by nt -1/-70 of *S. ramocissimus tuf1*	this study
pGWS2	pWHM3 containing the *Eco*RI/*Hin*dIII insert from pGWS1	this study
pGWS3	pWHM3-E containing the *Bgl*II/*Hin*dIII insert from pGWS1	this study
pGWS3-SD	pWHM3-E containing the *Bgl*II/*Hin*dIII insert from pGWS1-SD	this study
pGWS4	pSET152 containing the *Eco*RI/*Pst*I insert from pGWS3	this study

pGWS4-SD pSET152 containing the *Eco*RI/*Pst*I insert from pGWS3-SD this study

pGWS5 pIJ2925 containing the 580 bp *ssgA* PCR (ssg3/ssg2) product cloned *Eco*RI/*Bam*HI.

Table 1. Oligonucleotides and *ssgA* constructs. Nucleotide positions refer to the location of the primers in respect to the first nucleotide (+1) of the ATG translational start codon of *ssgA*. Underlined sequences indicate non-homologous sequences added to create restriction sites (in italics) at the ends of the PCR fragments.

	S. albus	*S. goldeniensis*	*S. griseus*	*S. netropsis*
S. albus	X	75.2	74.5	72.3
S. goldeniensis	71.3 (75.7)	X	77.5	75.7
S. griseus	66.2 (71.3)	78.7 (85.3)	X	83.3
S. netropsis	63.2 (70.6)	77.9 (83.8)	86.0 (90.4)	X

Table 2. DNA and deduced protein sequence homologies of *ssgA* homologues. Above the diagonal: DNA sequence identities (%). Below the diagonal: protein sequence identities (similarities between brackets).

References

Bibb, M.J., White, J., Ward, J.M., and Janssen, G.R. (1994) The mRNA for the 23S rRNA methylase encoded by the *ermE* gene of *Saccharopolyspora erythraea* is translated in the absence of a conventional ribosome-binding site. *Mol. Microbiol.* **14:** 533-45.

Bierman, M., R. Logan, K. Obrien, E.T. Seno, R.N. Rao, and Schoner, B.E. (1992) Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* **116:** 43-49.

Chater, K.F., and Losick, R. (1996) The mycelial life-style of *Streptomyces coelicolor* A3(2) and its relatives. In J.H. Shapiro and M. Dworkin (ed.), Bacteria as Multicellular Organisms. Oxford University Press, New York.

Dehottay, P., Dusart, J., De Meester, F., Joris, B., van Beeumen, J., Erpicum, T., Frere, J.-M., and Ghuysen, J.-M. (1987) Nucleotide sequence of the gene encoding the *Streptomyces albus* G ß-lactamase gene. *Eur. J. Biochem.* **166:** 345-350.

Devereux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12:** 387-395.

Ensign, J.C. (1988) Physiological regulation of sporulation of *Streptomyces griseus.* In Y. Okami, T. Beppu, and H. Ogawara (eds.), Biology of Actinomycetes 1988, pp. 308-315. Tokyo, Japan Scientific Societies Press.

Feinberg, A.P., and Vogelstein, B. (1983) A technique for radiolabeling of DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6-13.

Hobbs, G., Frazer, C.M., Gardner, D.C.J., Flett, F., and Oliver, S.G. (1989) Dispersed growth of *Streptomyces* in liquid culture. *Appl Microbiol Biotechnol* **31**:272-277.

Hopwood, D.A., Bibb, M.J., Chater, K.F., Kieser, T., Bruton, C.J., Kieser, H.M., Lydiate, D.J., Smith, C., Ward, J.M., and Schrempf, H. (1985) Genetic manipulation of *Streptomyces*: a laboratory manual. John Innes Foundation, Norwich, U.K.

Janssen, G.R., and Bibb, M.J. (1993) Derivatives of pUC18 that have *Bgl*II sites flanking a modified multiple cloning site and that retain the ability to identify recombinant clones by visual screening of *Escherichia coli* colonies. *Gene* **124:** 133-134.

Kawamoto, S., and Ensign, J.C. (1995a) Isolation of mutants of *Streptomyces griseus* that sporulate in nutrient rich media: cloning of DNA fragments that suppress the mutations. *Actinomycetologica* **9:** 124-135.

Kawamoto, S., and Ensign, J.C. (1995b) Cloning and characterization of a gene involved in regulation and sporulation and cell division in *Streptomyces griseus*. *Actinomycetologica* **9:** 136-151.

Kawamoto, S., Watanabe, H., Hesketh, A., Ensign, J.C., and Ochi, K. (1997) Expression of the *ssgA* gene product, associated with sporulation and cell division in *Streptomyces griseus*. *Microbiology* **143:** 1077-1086.

Kendrick, K., and Ensign, J.C. (1983) Sporulation of *Streptomyces griseus* in submerged culture. *J. Bacteriol.* **155:** 357-366.

Lutkenhaus, J., and Addinall, S.G. (1997) Bacterial cell division and the Z ring. *Annu Rev. Biochem.* **66:** 993-116.

MacNeil, D.J., Gewain, K.M., Ruby, C.L., Dezeny, G., Gibbons, P.H., and MacNeil, T. (1992) Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilising a novel integration vector. *Gene* **111:** 1-68.

McCarthy, A.J., and Williams, S.T. (1992) Actinomycetes as agents of biodegradation in the environment - a review. *Gene* **115:** 189-192.

McCormick, J.R., Su, E.P., Driks, A., and Losick, R. (1994) growth and viability of *Streptomyces coelicolor* mutant for the cell division gene *ftsZ*. *Mol. Microbiol.* **14:** 243-254.

Messing, J., Crea, R., and Seeburg, P.H. (1981) A system for shotgun DNA sequencing. *Nucleic Acids Res* **9:** 309-321.

Miyadoh, S. (1993) Research on antibiotic screening in Japan over the last decade: a producing microorganisms approach. *Actinomycetol* **7:** 100-106.

Redenbach, M., Kieser, H.M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H., and Hopwood, D.A. (1996) A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb *Streptomyces coelicolor* A3(2) chromosome. *Mol Microbiol.*

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Strohl, W.R. (1992) Compilation and analysis of DNA sequences associated with apparent streptomycete promoters. *Nucleic Acids Res* **20:** 961-974.

van Wezel, G.P., Vijgenboom, E., and Bosch, L. (1991) A comparative study of the ribosomal RNA operons of *Streptomyces coelicolor* A3(2) and sequence analysis of *rrnA*. *Nucleic Acids Res* **19:** 4399-4403.

Vara, J., Lewandowska-Skarbek, M., Wang, Y.-G., Donadio, S., and Hutchinson, C.R. (1989) Cloning of genes governing the deoxysugar portion of the erythromycin biosynthesis pathway in *saccharopolyspora erythraea* (*Streptomyces erythreus*). *J. Bacteriol.* **171:** 5872-5881.

Vijgenboom, E., Woudt, L.P., Heinstra, P.W.H., Rietveld, K., van Haarlem, J., van Wezel, G.P., Shochat, S., and Bosch, L. (1994) three *tuf*-like genes in the kirromycin producer *Streptomyces ramocissimus*. *Microbiology* **140:** 983-998.

Ward, J.M., Janssen, G.R., Kieser, T., Bibb, M.J., Buttner, M.J., and Bibb, M.J. (1986) Construction and characterisation of a series of multi-copy promoter-probe plasmid vectors for *Streptomyces* using the aminoglycoside phosphotransferase gene from Tn5 as indicator. *Mol Gen Genet* 203: 468-475.

Yanish-Perron, C., Vieira, J., and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13 mp18 and pUC19 vectors. *Gene* 33: 103 119.

Claims

1. A method for producing a filamentous bacterium showing reduced branching during growth, particularly growth in a liquid medium, comprising providing such a bacterium with the capability of having or expressing heterologous SsgA-activity, which activity in *Streptomyces Griseus* is encoded by an ssgA gene having at least the sequence

1 ATGCGCGAGTCGGTTCAAGCAGAGGTCATGATGAGCTTCCTCGTCTCCGA

51 GGAGCTCTCGTTCCGTATTCCGGTGGAGCTCCGATACGAGGTCGGCGATC

101 CGTATGCCATCCGGATGACGTTCCACCTTCCCGGCGATGCCCCTGTGACC

151 TGGGCGTTCGGCCGCGAGCTGCTGCTGGACGGGCTCAACAGCCCGAGCGG

201 CGACGGCGATGTGCACATCGGCCCGACCGAGCCCGAGGGCCTCGGAGATG

251 TCCACATCCGGCTCCAGGTCGGCGCGGACCGTGCGCTGTTCCGGGCGGGG

301 ACGGCACCGCTGGTGGCGTTCCTCGACCGGACGGACAAGCTCGTGCCGCT

351 CGGCCAGGAGCACACGCTGGGTGACTTCGACGGCAACCTGGAGGACGCAC

401 TGGGCCGCATCCTCGCCGAGGAGCAGAACGCCGGCTGA.

2. A method for producing a filamentous bacterium showing enhanced fragmentation during growth, particularly growth in a liquid medium, comprising providing such a bacterium with the capability of having or expressing heterologous SsgA-activity, which activity in *Streptomyces Griseus* is encoded by an *ssgA* gene having the sequence

```
1   ATGCGCGAGTCGGTTCAAGCAGAGGTCATGATGAGCTTCCTCGTCTCCGA

51  GGAGCTCTCGTTCCGTATTCCGGTGGAGCTCCGATACGAGGTCGGCGATC

101 CGTATGCCATCCGGATGACGTTCCACCTTCCCGGCGATGCCCCTGTGACC

151 TGGGCGTTCGGCCGCGAGCTGCTGCTGGACGGGCTCAACAGCCCGAGCGG

201 CGACGGCGATGTGCACATCGGCCCGACCGAGCCCGAGGGCCTCGGAGATG

251 TCCACATCCGGCTCCAGGTCGGCGCGGACCGTGCGCTGTTCCGGGCGGGG

301 ACGGCACCGCTGGTGGCGTTCCTCGACCGGACGGACAAGCTCGTGCCGCT

351 CGGCCAGGAGCACACGCTGGGTGACTTCGACGGCAACCTGGAGGACGCAC

401 TGGGCCGCATCCTCGCCGAGGAGCAGAACGCCGGCTGA
```

3. A method according to claim 1 or 2, whereby said additional SsgA-activity is provided by transfecting or transforming said filamentous bacterium with additional genetic information encoding said activity.

4. A method according to claim 3, whereby said additional genetic information comprises an *ssgA* gene or a derivative or a fragment thereof encoding similar SsgA-activity.

5. A method according to claim 4, whereby said *ssgA* gene is derived from an actinomycete.

6. A method according to claim 4, whereby said gene is derived from a streptomycete.

7. A method according to claim 5, whereby said gene is derived from *Streptomyces griseus*, *Streptomyces collinus*, *Streptomyces albus*, *Streptomyces goldeniensis* or *Streptomyces netropsis*.

8. A method according to any one of claims 3-7, whereby said additional genetic information is integrated into the bacterial genome.

9. A method according to any one of claims 3-8, whereby said additional genetic information is part of an episomal element.

10. A method according to any one of the aforegoing claims, whereby said filamentous bacterium does not have significant endogenous ssgA-activity.

11. A method according to any one of the aforegoing claims wherein said ssgA-activity is inducible or repressible with a signal.

12. A method according to any one of the aforegoing claims wherein said filamentous bacterium is an Actinomyces.

13. A method according to claim 12, wherein said bacterium is a *Streptomyces*.

14. A method according to any one of the aforegoing claims whereby said bacterium produces a useful product.

15. A method according to claim 14 wherein said useful product is an antibiotic.

16. A method according to claim 14, whereby said useful product is a protein.

17. A method according to claim 16 whereby said protein is heterologous to said bacterium.

18. A method according to claim 16 or 17, whereby said protein is expressed from a vector encoding said protein present in said bacterium.

19. A method according to claim 16, 17 or 18, whereby said protein is secreted by said bacterium.

20. A filamentous bacterium obtainable by a method according to any one of the aforegoing claims.

21. A filamentous bacterium according to claim 20, which is an actinomycete, preferably a *Streptomyces*.

22. A method for producing an antibiotic or a useful protein comprising culturing a filamentous bacterium according to claim 19 or 21 and harvesting said antibiotic or protein from said culture.

23. A method according to claim 22 whereby said culturing is submerged culture.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Rijksuniversiteit Leiden
(B) STREET: Wassenaarseweg 72
(C) CITY: Leiden
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): 2300 RA

(ii) TITLE OF INVENTION: Reducing branching and enhancing fragmentation in culturing filamentous microorganisms

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptomyces griseus
(B) STRAIN: ATTC 23345

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..408
(D) OTHER INFORMATION: /product= "SsgA"
/gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ATG AGC TTC CTC GTC TCC GAG GAG CTC TCG TTC CGT ATT CCG GTG GAG        48
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
  1               5                  10                  15

CTC CGA TAC GAG GTC GGC GAT CCG TAT GCC ATC CGG ATG ACG TTC CAC        96
Leu Arg Tyr Glu Val Gly Asp Pro Tyr Ala Ile Arg Met Thr Phe His
            20                  25                  30
```

```
CTT CCC GGC GAT GCC CCT GTG ACC TGG GCG TTC GGC CGC GAG CTG CTG      144
Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
         35                  40                  45

CTG GAC GGG CTC AAC AGC CCG AGC GGC GAC GGC GAT GTG CAC ATC GGC      192
Leu Asp Gly Leu Asn Ser Pro Ser Gly Asp Gly Asp Val His Ile Gly
     50                  55                  60

CCG ACC GAG CCC GAG GGC CTC GGA GAT GTC CAC ATC CGG CTC CAG GTC      240
Pro Thr Glu Pro Glu Gly Leu Gly Asp Val His Ile Arg Leu Gln Val
 65                  70                  75                  80

GGC GCG GAC CGT GCG CTG TTC CGG GCG GGG ACG GCA CCG CTG GTG GCG      288
Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Thr Ala Pro Leu Val Ala
                 85                  90                  95

TTC CTC GAC CGG ACG GAC AAG CTC GTG CCG CTC GGC CAG GAG CAC ACG      336
Phe Leu Asp Arg Thr Asp Lys Leu Val Pro Leu Gly Gln Glu His Thr
            100                 105                 110

CTG GGT GAC TTC GAC GGC AAC CTG GAG GAC GCA CTG GGC CGC ATC CTC      384
Leu Gly Asp Phe Asp Gly Asn Leu Glu Asp Ala Leu Gly Arg Ile Leu
        115                 120                 125

GCC GAG GAG CAG AAC GCC GGC TG                                       408
Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
 1               5                  10                  15

Leu Arg Tyr Glu Val Gly Asp Pro Tyr Ala Ile Arg Met Thr Phe His
            20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

Leu Asp Gly Leu Asn Ser Pro Ser Gly Asp Gly Asp Val His Ile Gly
    50                  55                  60
```

```
Pro Thr Glu Pro Glu Gly Leu Gly Asp Val His Ile Arg Leu Gln Val
 65                  70                  75                  80

Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Thr Ala Pro Leu Val Ala
            85                  90                  95

Phe Leu Asp Arg Thr Asp Lys Leu Val Pro Leu Gly Gln Glu His Thr
        100                 105                 110

Leu Gly Asp Phe Asp Gly Asn Leu Glu Asp Ala Leu Gly Arg Ile Leu
    115                 120                 125

Ala Glu Glu Gln Asn Ala Gly
130                 135
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptomyces albus G
(B) STRAIN: ATCC 3004

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..408
(D) OTHER INFORMATION: /product= "SsgA"
/gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
ATG AGC TTC CTC GTC TCC GAG GAG CTC GCC TTC CGC ATC CCG GTG GAG      48
Met Ser Phe Leu Val Ser Glu Glu Leu Ala Phe Arg Ile Pro Val Glu
 1               5                  10                  15

CTG CGG TAC GAG ACC GTC GAT CCG TAC GCG GTG CGG CTG ACG TTC CAC      96
Leu Arg Tyr Glu Thr Val Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

CTC CCC GGA GAC GCC CCG GTC ACC TGG GTC TTC GGG CGT GAA CTG CTG     144
Leu Pro Gly Asp Ala Pro Val Thr Trp Val Phe Gly Arg Glu Leu Leu
        35                  40                  45
```

```
GTC GAG GGA GTC CTG GAC GCC GCG GGC GAC GGC GAC GTC CGG GTC TGC      192
Val Glu Gly Val Leu Asp Ala Ala Gly Asp Gly Asp Val Arg Val Cys
     50                  55                  60

CCG GTG GGG CAG ACG GCC ACC AGG GAG GTG CAC ATC ACC CTC CAG GTC      240
Pro Val Gly Gln Thr Ala Thr Arg Glu Val His Ile Thr Leu Gln Val
 65                  70                  75                  80

GGC TCC GAG CAG GCG CTC TTC CGC GTC GGC AAG GCG CCG CTG CTC GCC      288
Gly Ser Glu Gln Ala Leu Phe Arg Val Gly Lys Ala Pro Leu Leu Ala
                 85                  90                  95

TTC CTC GAC CGC ACC GAC CAG GGC TTG TCG CTC GGC AGC GAG CGG GCA      336
Phe Leu Asp Arg Thr Asp Gln Gly Leu Ser Leu Gly Ser Glu Arg Ala
            100                 105                 110

CAC GCC GAC TTC GAC AGC CAC CTC GAC GAC GCT CTG AAC CGC AGC CTC      384
His Ala Asp Phe Asp Ser His Leu Asp Asp Ala Leu Asn Arg Ser Leu
        115                 120                 125

GCC GAG GAG CAG AGC GCC GGC TG                                      408
Ala Glu Glu Gln Ser Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ala Phe Arg Ile Pro Val Glu
  1               5                  10                  15

Leu Arg Tyr Glu Thr Val Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Val Phe Gly Arg Glu Leu Leu
        35                  40                  45

Val Glu Gly Val Leu Asp Ala Ala Gly Asp Gly Asp Val Arg Val Cys
    50                  55                  60

Pro Val Gly Gln Thr Ala Thr Arg Glu Val His Ile Thr Leu Gln Val
 65                  70                  75                  80

Gly Ser Glu Gln Ala Leu Phe Arg Val Gly Lys Ala Pro Leu Leu Ala
```

```
                85                  90                  95

Phe Leu Asp Arg Thr Asp Gln Gly Leu Ser Leu Gly Ser Glu Arg Ala
            100                 105                 110

His Ala Asp Phe Asp Ser His Leu Asp Asp Ala Leu Asn Arg Ser Leu
        115                 120                 125

Ala Glu Glu Gln Ser Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 408 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Streptomyces goldiniensis
- (B) STRAIN: ATCC 21386

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..408
- (D) OTHER INFORMATION: /product= "SsgA" /gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
ATG AGC TTC CTC GTC TCG GAA GAA CTC TCC TTC CGT ATT CCG GTG GAG      48
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
  1               5                  10                  15

CTG CGT TAC GAG ACC TGT GAT CCC TAC GCC GTG CGG CTG ACC TTT CAT      96
Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

CTG CCC GGA GAT GCC CCG GTG ACC TGG GCG TTC GGG CGG GAG TTG CTC     144
Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

ATC GAC GGA GGT CCG CGG CCG TGC GGG GAC GGG GAC GTC CAC ATC GCG     192
Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala
    50                  55                  60
```

```
CCC GCC GAC CCG GAG ACG TTC GGC GAG GTC CTG ATC CGC CTG CAG GTC      240
Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val
 65                  70                  75                  80

GGG AGC GAC CAG GCG ATG TTC CGG GTC GGC ACG GCG CCG CTG GTG GCC      288
Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala
                 85                  90                  95

TTC CTG GAC CGC ACG GAC AAG ATC GTG CCG CTG GGG CAG GAG CGT TCC      336
Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser
            100                 105                 110

CTC GCC GAC TTC GAC GCC CTG CTC GAC GAG GCG CTG GAC CGC ATC CTG      384
Leu Ala Asp Phe Asp Ala Leu Leu Asp Glu Ala Leu Asp Arg Ile Leu
        115                 120                 125

GCC GAG GAG CAG AAC GCC GGC TG                                       408
Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
 1               5                  10                  15

Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala
    50                  55                  60

Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val
65                  70                  75                  80

Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala
                85                  90                  95

Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser
            100                 105                 110
```

```
Leu Ala Asp Phe Asp Ala Leu Leu Asp Glu Ala Leu Asp Arg Ile Leu
      115                 120                 125

Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptomyces netropsis
(B) STRAIN: ATCC 23940

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..408
(D) OTHER INFORMATION: /product= "SsgA"
/gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
ATG AGC TTC CTC GTC TCC GAG GAG CTC TCC TTC AAG ATC CCA GTC GAA      48
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Lys Ile Pro Val Glu
  1               5                  10                  15

CTG CGA TAC GAG ACC CGG GAT CCC TAC GCG GTG CGG ATG ACC TTC CAC      96
Leu Arg Tyr Glu Thr Arg Asp Pro Tyr Ala Val Arg Met Thr Phe His
             20                  25                  30

CTC CCC GGA GAC GCG CCT GTG ACC TGG GCG TTC GGC CGG GAG CTG CTG     144
Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
         35                  40                  45

CTC GAC GGG ATC AAC CGC CCG AGC GGC GAC GGC GAC GTC CAC ATC GCC     192
Leu Asp Gly Ile Asn Arg Pro Ser Gly Asp Gly Asp Val His Ile Ala
     50                  55                  60

CCG ACC GAC CCC GAG GGC CTG TCG GAC GTC TCC ATC CGG CTC CAG GTG     240
Pro Thr Asp Pro Glu Gly Leu Ser Asp Val Ser Ile Arg Leu Gln Val
 65                  70                  75                  80
```

```
GGC GCG GAC CGC GCC CTC TTC CGT GCA GGC GCC CCG CCG CTG GTC GCC       288
Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Ala Pro Pro Leu Val Ala
                 85                  90                  95

TTC CTC GAC CGC ACG GAC AAG TCG GTG CCG CTC GGT CAG GAA CAG ACT       336
Phe Leu Asp Arg Thr Asp Lys Ser Val Pro Leu Gly Gln Glu Gln Thr
            100                 105                 110

CTG GGT GAC TTC GAG GAC AGC CTG GAG GCC GCG CTC GGC AAG ATC CTC       384
Leu Gly Asp Phe Glu Asp Ser Leu Glu Ala Ala Leu Gly Lys Ile Leu
        115                 120                 125

GCC GAG GAG CAG AAC GCC GGC TG                                        408
Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Lys Ile Pro Val Glu
 1               5                  10                  15

Leu Arg Tyr Glu Thr Arg Asp Pro Tyr Ala Val Arg Met Thr Phe His
            20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

Leu Asp Gly Ile Asn Arg Pro Ser Gly Asp Gly Asp Val His Ile Ala
    50                  55                  60

Pro Thr Asp Pro Glu Gly Leu Ser Asp Val Ser Ile Arg Leu Gln Val
65                  70                  75                  80

Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Ala Pro Pro Leu Val Ala
                85                  90                  95

Phe Leu Asp Arg Thr Asp Lys Ser Val Pro Leu Gly Gln Glu Gln Thr
            100                 105                 110

Leu Gly Asp Phe Glu Asp Ser Leu Glu Ala Ala Leu Gly Lys Ile Leu
        115                 120                 125
```

Ala Glu Glu Gln Asn Ala Gly
130 135

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGCGAATTCG AACAGCTACG TGGCGAAGTC GCCA 34

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTGGGATCCG TGCTCGCGGC GCTGGTCGTC TC 32

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGGAATTCCA TATGCGCGAG TCGGTTCAAG CA 32

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCGGTCAGCC GGCGTTCTGC TCCTC 25

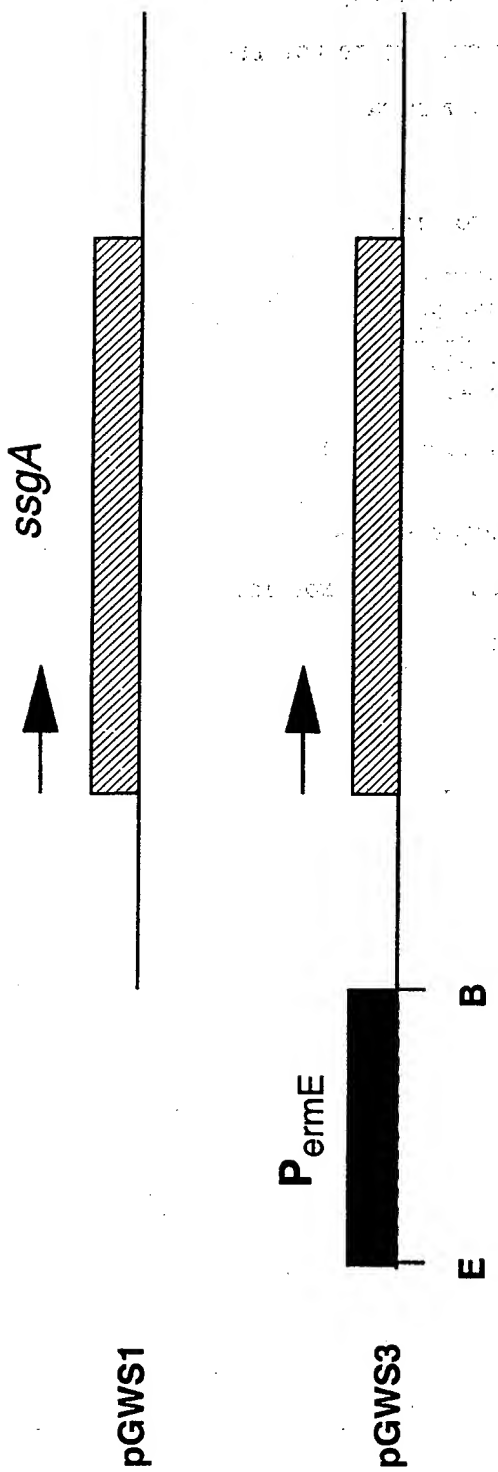


Figure 1

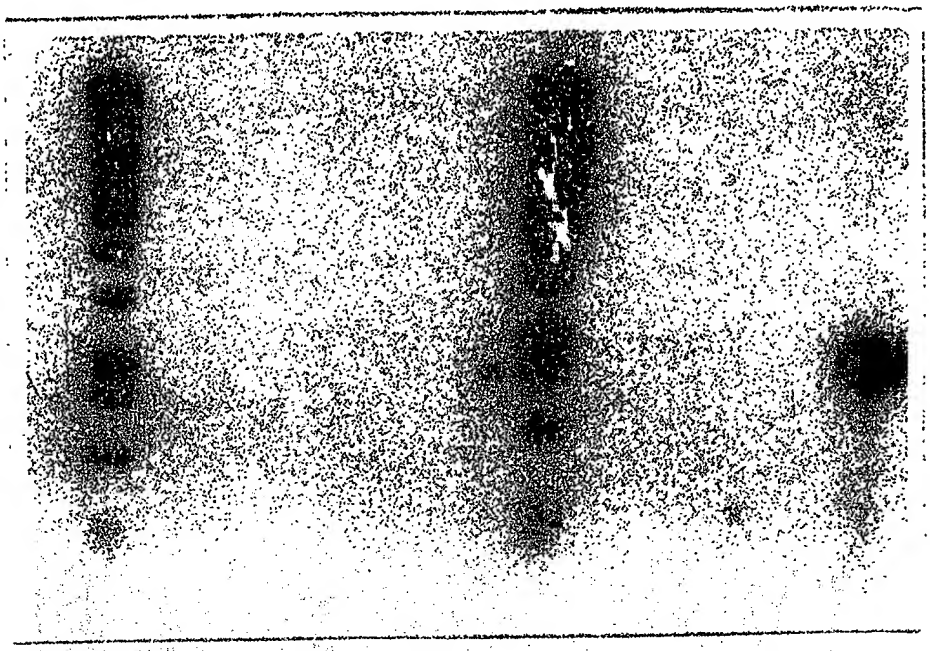


Figure 2A

Figure 2B

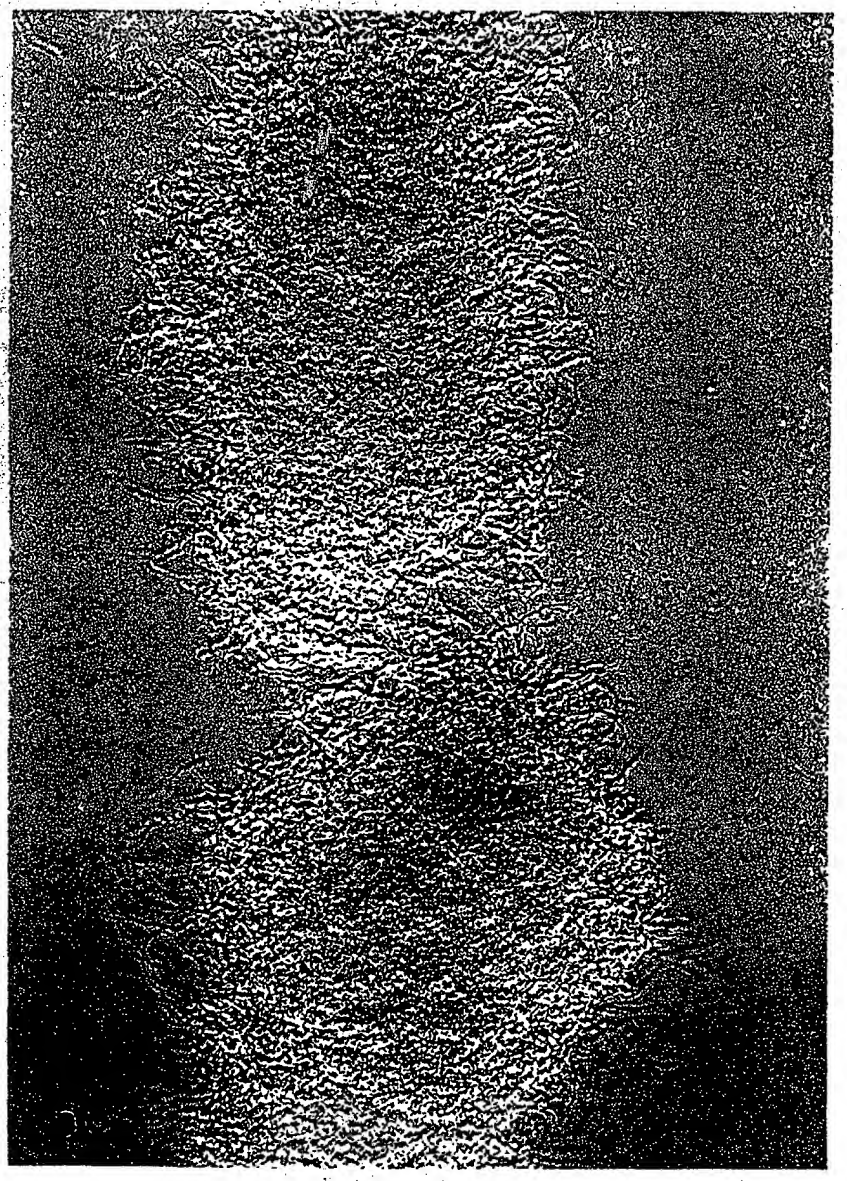
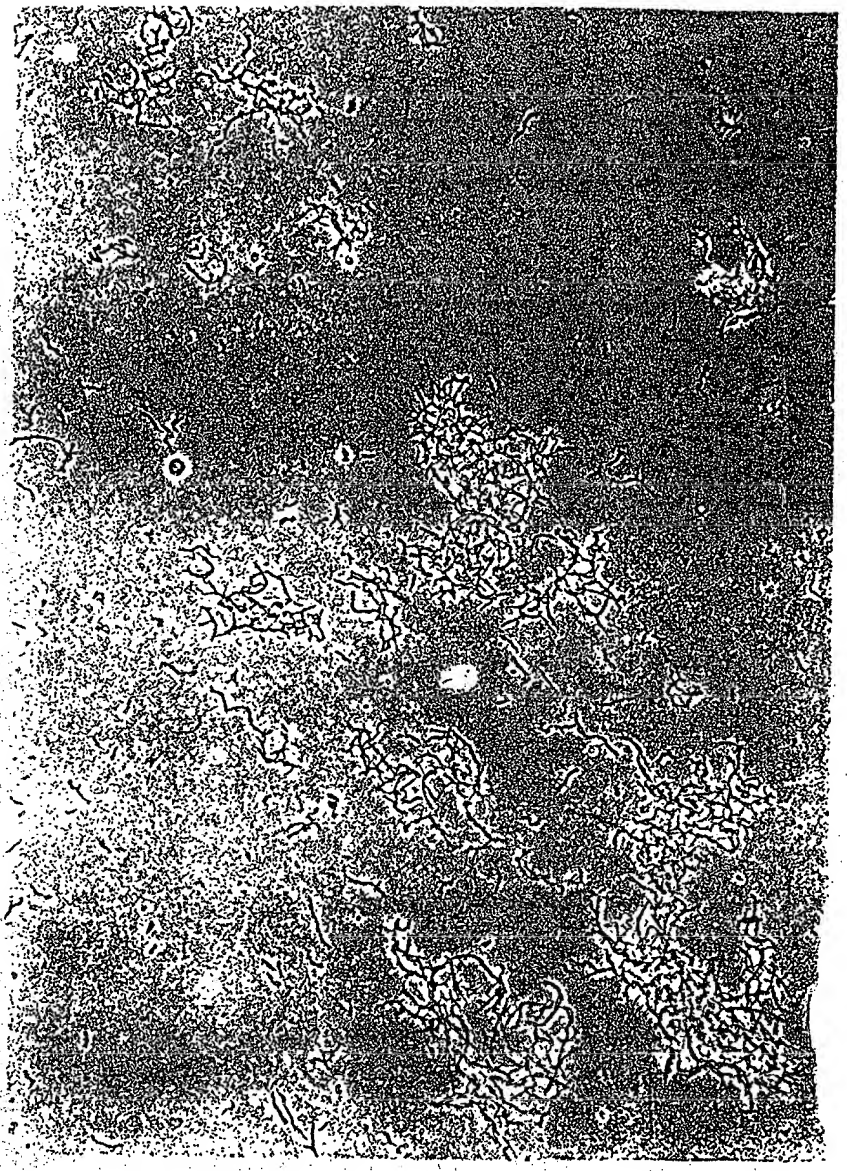


Figure 3A



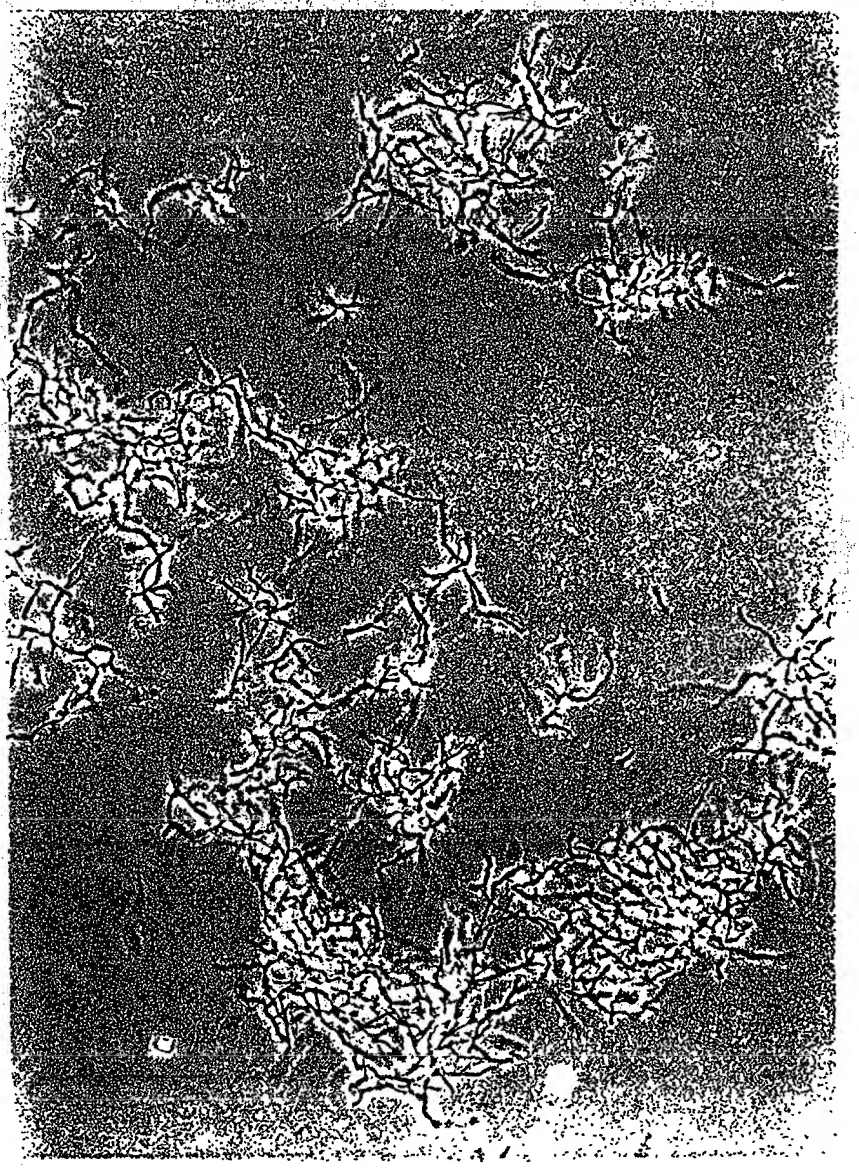


Figure 4

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Rijksuniversiteit Leiden
(B) STREET: Wassenaarseweg 72
(C) CITY: Leiden
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): 2300 RA

(ii) TITLE OF INVENTION: Reducing branching and enhancing fragmentation in culturing filamentous microorganisms

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptomyces griseus
(B) STRAIN: ATTC 23345

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..408
(D) OTHER INFORMATION: /product= "SsgA"
/gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ATG AGC TTC CTC GTC TCC GAG GAG CTC TCG TTC CGT ATT CCG GTG GAG        48
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
 1               5                  10                  15

CTC CGA TAC GAG GTC GGC GAT CCG TAT GCC ATC CGG ATG ACG TTC CAC        96
Leu Arg Tyr Glu Val Gly Asp Pro Tyr Ala Ile Arg Met Thr Phe His
            20                  25                  30
```

Figure 5

```
CTT CCC GGC GAT GCC CCT GTG ACC TGG GCG TTC GGC CGC GAG CTG CTG      144
Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

CTG GAC GGG CTC AAC AGC CCG AGC GGC GAC GGC GAT GTG CAC ATC GGC      192
Leu Asp Gly Leu Asn Ser Pro Ser Gly Asp Gly Asp Val His Ile Gly
     50                  55                  60

CCG ACC GAG CCC GAG GGC CTC GGA GAT GTC CAC ATC CGG CTC CAG GTC      240
Pro Thr Glu Pro Glu Gly Leu Gly Asp Val His Ile Arg Leu Gln Val
 65                  70                  75                  80

GGC GCG GAC CGT GCG CTG TTC CGG GCG GGG ACG GCA CCG CTG GTG GCG      288
Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Thr Ala Pro Leu Val Ala
                 85                  90                  95

TTC CTC GAC CGG ACG GAC AAG CTC GTG CCG CTC GGC CAG GAG CAC ACG      336
Phe Leu Asp Arg Thr Asp Lys Leu Val Pro Leu Gly Gln Glu His Thr
            100                 105                 110

CTG GGT GAC TTC GAC GGC AAC CTG GAG GAC GCA CTG GGC CGC ATC CTC      384
Leu Gly Asp Phe Asp Gly Asn Leu Glu Asp Ala Leu Gly Arg Ile Leu
        115                 120                 125

GCC GAG GAG CAG AAC GCC GGC TG                                       408
Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
  1               5                  10                  15

Leu Arg Tyr Glu Val Gly Asp Pro Tyr Ala Ile Arg Met Thr Phe His
             20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
         35                  40                  45

Leu Asp Gly Leu Asn Ser Pro Ser Gly Asp Gly Asp Val His Ile Gly
     50                  55                  60
```

Figure 5 continued

```
Pro Thr Glu Pro Glu Gly Leu Gly Asp Val His Ile Arg Leu Gln Val
 65                  70                  75                  80

Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Thr Ala Pro Leu Val Ala
                85                  90                  95

Phe Leu Asp Arg Thr Asp Lys Leu Val Pro Leu Gly Gln Glu His Thr
            100                 105                 110

Leu Gly Asp Phe Asp Gly Asn Leu Glu Asp Ala Leu Gly Arg Ile Leu
        115                 120                 125

Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 408 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Streptomyces albus G
- (B) STRAIN: ATCC 3004

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..408
- (D) OTHER INFORMATION: /product= "SsgA" /gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
ATG AGC TTC CTC GTC TCC GAG GAG CTC GCC TTC CGC ATC CCG GTG GAG      48
Met Ser Phe Leu Val Ser Glu Glu Leu Ala Phe Arg Ile Pro Val Glu
  1               5                  10                  15

CTG CGG TAC GAG ACC GTC GAT CCG TAC GCG GTG CGG CTG ACG TTC CAC      96
Leu Arg Tyr Glu Thr Val Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

CTC CCC GGA GAC GCC CCG GTC ACC TGG GTC TTC GGG CGT GAA CTG CTG     144
Leu Pro Gly Asp Ala Pro Val Thr Trp Val Phe Gly Arg Glu Leu Leu
        35                  40                  45
```

Figure 5 continued

```
GTC GAG GGA GTC CTG GAC GCC GCG GGC GAC GGC GAC GTC CGG GTC TGC      192
Val Glu Gly Val Leu Asp Ala Ala Gly Asp Gly Asp Val Arg Val Cys
     50                  55                  60

CCG GTG GGG CAG ACG GCC ACC AGG GAG GTG CAC ATC ACC CTC CAG GTC      240
Pro Val Gly Gln Thr Ala Thr Arg Glu Val His Ile Thr Leu Gln Val
 65                  70                  75                  80

GGC TCC GAG CAG GCG CTC TTC CGC GTC GGC AAG GCG CCG CTG CTC GCC      288
Gly Ser Glu Gln Ala Leu Phe Arg Val Gly Lys Ala Pro Leu Leu Ala
                85                  90                  95

TTC CTC GAC CGC ACC GAC CAG GGC TTG TCG CTC GGC AGC GAG CGG GCA      336
Phe Leu Asp Arg Thr Asp Gln Gly Leu Ser Leu Gly Ser Glu Arg Ala
           100                 105                 110

CAC GCC GAC TTC GAC AGC CAC CTC GAC GAC GCT CTG AAC CGC AGC CTC      384
His Ala Asp Phe Asp Ser His Leu Asp Asp Ala Leu Asn Arg Ser Leu
       115                 120                 125

GCC GAG GAG CAG AGC GCC GGC TG                                       408
Ala Glu Glu Gln Ser Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ala Phe Arg Ile Pro Val Glu
  1               5                  10                  15

Leu Arg Tyr Glu Thr Val Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Val Phe Gly Arg Glu Leu Leu
        35                  40                  45

Val Glu Gly Val Leu Asp Ala Ala Gly Asp Gly Asp Val Arg Val Cys
     50                  55                  60

Pro Val Gly Gln Thr Ala Thr Arg Glu Val His Ile Thr Leu Gln Val
 65                  70                  75                  80

Gly Ser Glu Gln Ala Leu Phe Arg Val Gly Lys Ala Pro Leu Leu Ala
```

Figure 5 continued

```
                85                  90                  95
Phe Leu Asp Arg Thr Asp Gln Gly Leu Ser Leu Gly Ser Glu Arg Ala
            100                 105                 110

His Ala Asp Phe Asp Ser His Leu Asp Asp Ala Leu Asn Arg Ser Leu
        115                 120                 125

Ala Glu Glu Gln Ser Ala Gly
    130             135
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptomyces goldiniensis
(B) STRAIN: ATCC 21386

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..408
(D) OTHER INFORMATION: /product= "SsgA"
/gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
ATG AGC TTC CTC GTC TCG GAA GAA CTC TCC TTC CGT ATT CCG GTG GAG      48
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
  1               5                  10                  15

CTG CGT TAC GAG ACC TGT GAT CCC TAC GCC GTG CGG CTG ACC TTT CAT      96
Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

CTG CCC GGA GAT GCC CCG GTG ACC TGG GCG TTC GGG CGG GAG TTG CTC     144
Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

ATC GAC GGA GGT CCG CGG CCG TGC GGG GAC GGG GAC GTC CAC ATC GCG     192
Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala
    50                  55                  60
```

Figure 5 continued

```
CCC GCC GAC CCG GAG ACG TTC GGC GAG GTC CTG ATC CGC CTG CAG GTG        240
Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val
 65                  70                  75                  80

GGG AGC GAC CAG GCG ATG TTC CGG GTC GGC ACG GCG CCG CTG GTG GCC        288
Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala
                 85                  90                  95

TTC CTG GAC CGC ACG GAC AAG ATC GTG CCG CTG GGG CAG GAG CGT TCC        336
Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser
            100                 105                 110

CTC GCC GAC TTC GAC GCC CTG CTC GAC GAG GCG CTG GAC CGC ATC CTG        384
Leu Ala Asp Phe Asp Ala Leu Leu Asp Glu Ala Leu Asp Arg Ile Leu
        115                 120                 125

GCC GAG GAG CAG AAC GCC GGC TG                                         408
Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Arg Ile Pro Val Glu
  1               5                  10                  15

Leu Arg Tyr Glu Thr Cys Asp Pro Tyr Ala Val Arg Leu Thr Phe His
            20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

Ile Asp Gly Gly Pro Arg Pro Cys Gly Asp Gly Asp Val His Ile Ala
    50                  55                  60

Pro Ala Asp Pro Glu Thr Phe Gly Glu Val Leu Ile Arg Leu Gln Val
 65                  70                  75                  80

Gly Ser Asp Gln Ala Met Phe Arg Val Gly Thr Ala Pro Leu Val Ala
                 85                  90                  95

Phe Leu Asp Arg Thr Asp Lys Ile Val Pro Leu Gly Gln Glu Arg Ser
            100                 105                 110
```

Figure 5 continued

```
Leu Ala Asp Phe Asp Ala Leu Leu Asp Glu Ala Leu Asp Arg Ile Leu
        115                 120                 125

Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Streptomyces netropsis
(B) STRAIN: ATCC 23940

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..408
(D) OTHER INFORMATION: /product= "SsgA"
/gene= "ssgA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
ATG AGC TTC CTC GTC TCC GAG GAG CTC TCC TTC AAG ATC CCA GTC GAA      48
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Lys Ile Pro Val Glu
 1               5                  10                  15

CTG CGA TAC GAG ACC CGG GAT CCC TAC GCG GTG CGG ATG ACC TTC CAC      96
Leu Arg Tyr Glu Thr Arg Asp Pro Tyr Ala Val Arg Met Thr Phe His
            20                  25                  30

CTC CCC GGA GAC GCG CCT GTG ACC TGG GCC TTC GGC CGG GAG CTG CTG     144
Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

CTC GAC GGG ATC AAC CGC CCG AGC GGC GAC GGC GAC GTC CAC ATC GCC     192
Leu Asp Gly Ile Asn Arg Pro Ser Gly Asp Gly Asp Val His Ile Ala
    50                  55                  60

CCG ACC GAC CCC GAG GGC CTG TCG GAC GTC TCC ATC CGG CTC CAG GTG     240
Pro Thr Asp Pro Glu Gly Leu Ser Asp Val Ser Ile Arg Leu Gln Val
65                  70                  75                  80
```

Figure 5 continued

```
GGC GCG GAC CGC GCC CTC TTC CGT GCA GGC GCC CCG CCG CTG GTC GCC      288
Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Ala Pro Pro Leu Val Ala
                85                  90                  95

TTC CTC GAC CGC ACG GAC AAG TCG GTG CCG CTC GGT CAG GAA CAG ACT      336
Phe Leu Asp Arg Thr Asp Lys Ser Val Pro Leu Gly Gln Glu Gln Thr
            100                 105                 110

CTG GGT GAC TTC GAG GAC AGC CTG GAG GCC GCG CTC GGC AAG ATC CTC      384
Leu Gly Asp Phe Glu Asp Ser Leu Glu Ala Ala Leu Gly Lys Ile Leu
        115                 120                 125

GCC GAG GAG CAG AAC GCC GGC TG                                       408
Ala Glu Glu Gln Asn Ala Gly
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Ser Phe Leu Val Ser Glu Glu Leu Ser Phe Lys Ile Pro Val Glu
 1               5                  10                  15

Leu Arg Tyr Glu Thr Arg Asp Pro Tyr Ala Val Arg Met Thr Phe His
            20                  25                  30

Leu Pro Gly Asp Ala Pro Val Thr Trp Ala Phe Gly Arg Glu Leu Leu
        35                  40                  45

Leu Asp Gly Ile Asn Arg Pro Ser Gly Asp Gly Asp Val His Ile Ala
    50                  55                  60

Pro Thr Asp Pro Glu Gly Leu Ser Asp Val Ser Ile Arg Leu Gln Val
65                  70                  75                  80

Gly Ala Asp Arg Ala Leu Phe Arg Ala Gly Ala Pro Pro Leu Val Ala
                85                  90                  95

Phe Leu Asp Arg Thr Asp Lys Ser Val Pro Leu Gly Gln Glu Gln Thr
            100                 105                 110

Leu Gly Asp Phe Glu Asp Ser Leu Glu Ala Ala Leu Gly Lys Ile Leu
        115                 120                 125
```

Figure 5 continued

Ala Glu Glu Gln Asn Ala Gly
130 135

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGCGAATTCG AACAGCTACG TGGCGAAGTC GCCA 34

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTGGGATCCG TGCTCGCGGC GCTGGTCGTC TC 32

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

Figure 5 continued

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGGAATTCCA TATGCGCGAG TCGGTTCAAG CA 32

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ssg4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCGGTCAGCC GGCGTTCTGC TCCTC 25

Figure 5 continued